# REMARKS

Applicants request reconsideration in light of the above amendment and following comments.

## 1. Status of the Claims

**Claims canceled:** Claims 10, 17-20, and 24-26

**Claims pending:** Claims 1-9, 11-16, 21-23, 27-30

**Claims allowed:** None

**Claims rejected:** Claims 10 and 14

**Claims withdrawn:** Claims 1-9, 11-13, 15-16, 21-23, and 27-30

Applicants cancel claims 10, 17-20, and 24-26 without prejudice or disclaimer of any subject matter. Applicants reserve the right to file a continuation or divisional application on any subject matter canceled by way of amendments.

## 2. Support for the Amendments

Claim 14 is amended to remove the product-by-process feature, thereby clarifying the claims. The amendments to claim 14 and the other withdrawn claims are made without prejudice or disclaimer of any subject matter.

Following entry of the amendment, claims 1-9 , 12-16, and 27-30 now depend from claim 11. Claim 11 stands as the only independent claim. Claim 11 is related to claims 12-16 as genus/species, respectively, so amendment of claims 12-16 to depend from claim 11 does not introduce impermissible new matter.

Claims 21-23 are amended to depend from claim 1, which itself depends from claim 11. Claim 1 is related to claims 21-23 as genus/species, so amendment of claims 21-23 to depend from claim 11 does not introduce impermissible new matter.

The amendments do not add subject matter that is unsupported by the specification.

## 3. Acceptance of the Drawings

Applicants note with appreciation the indication that the drawings submitted March 11, 2005, are deemed acceptable.

**4. Acknowledgement of Certified Priority Documents**

Applicants note with appreciation the indication that copies of the certified priority documents have been received in this application.

**5. Acknowledgement of Information Disclosure Statements**

Applicants note with appreciation the acknowledgement of the Information Disclosure Statements (IDS) submitted March 11, 2005; May 2, 2008; and March 27, 2009. Applicants request acknowledgement of the IDS filed July 20, 2009.

**6. Summary of the Contents of an Interview**

Applicants express appreciation to the Examiner for conducting telephone interviews with Applicants' counsel on October 19 and 22, 2009. During the interviews, the Examiner agreed to vacate formally the Notice of Non-Responsive Amendment mailed July 3, 2009. Applicants further requested rejoinder of withdrawn claim 11, as amended in the Amendment and Response under 37 C.F.R. § 1.111, filed herewith. The Examiner denied Applicants' request for rejoinder. Applicants accordingly file herewith a Petition under 37 C.F.R. § 1.144, requesting rejoinder of claim 11.

During the interview, the Examiner indicated that the withdrawn process claims likely contain allowable subject matter, for which Applicants express their appreciation. The Examiner also indicated that claim 11, as amended, "lacks enabling disclosure as to the structure of the claimed product(s)." *See* Interview Summary, at page 2.

The allegation that claim 11 does not comply with the enablement requirement of 35 U.S.C. § 112, first paragraph, is without merit and should be withdrawn. The specification discloses that the claimed composition can be made, for example, by transesterifying (a) 50-100 parts by weight of one or more fungus-produced oils/fats or triglycerides containing at least 20% of polyunsaturated fatty acids containing 20 or more carbons and two or more double bonds, and (b) no more than 50 parts by weight of one or more vegetable oils/fats or triglycerides, using a 1,3-position specific type lipase. The Office provides no reasoning or evidence why the skilled artisan would doubt this objectively enabling disclosure. *See In re Cortright*, 165 F.3d 1353,

1357, 49 USPQ2d 1464 (Fed. Cir. 1999). The allegation accordingly is unsubstantiated and should be withdrawn.

## 7. Rejections under 35 U.S.C. § 102(b)

The Office rejects claims 10 and 14 under 35 U.S.C. § 102(b) as allegedly anticipated by each of:

- JP 10-290699 ("**D1**");
- Tane et al., 46 J. JPN. OIL CHEM. SOC. 785, (1997) ("**D2**");
- JP 2000-004894 ("**D3**");
- Liu et al., 75 *JAOCS* 507(1998) ("**D7**"); and
- EP 0 965 578 (1999) ("**Akimoto**").

The Office alleges that each of the references above teach each and every feature of the claimed invention. The Office cites *In re Brown*, 459 F.2d 532 (CCPA 1972) and *In re Best*, 562 F.2d 1252 (CCPA 1977) for the proposition that the process for producing a claimed compound or composition is immaterial to the patentability of the compound or composition, absent evidence to the contrary.

Applicants traverse the rejections. Applicants do not dispute the holdings in *Brown* and *Best*. For anticipation, however, a single prior art reference must teach each and every element of the claimed invention, either explicitly or inherently. *See, e.g., Verdegaal Bros. v. Union Oil Co. Cal.*, 814 F.2d 628, 631, 2 USPQ2d 1051, 1053 (Fed. Cir. 1987). If the teaching is inherent, the Office ***must*** establish beyond probabilities or possibilities that the allegedly inherent properties occur in the cited art. *See, e.g., In re Oelrich*, 666 F.2d 578, 581-82 (CCPA 1981); *see also Ex parte Whalen*, 89 U.S.P.Q.2d 1078, 1083 (Bd. Pat. App. & Int. 2008) (precedential) ("even if some of the [prior art] compositions encompassed by Evans' broad disclosure might have a viscosity of 150 cSt at 40°C, that possibility is not adequate to support a finding of inherent anticipation."). In the present case, none of the cited references teaches each and every feature of the claimed invention, explicitly or inherently, for the following reasons.

To expedite prosecution, Applicants provide machine translations of Japanese published applications D1 and D3 (*see* **EXHIBIT 1** and **EXHIBIT 2**, respectively). It is the ***Office's*** responsibility, however, to obtain full translations of D1, D2, and D3, should an appeal be

necessary in this application. *See Ex parte Bonfils*, 64 USPQ2d 1456 (Bd. Pat. App. Int. 2002). Full translations of D1, D2, and D3 also are requested in the concurrently filed Petition under 37 C.F.R. § 1.144.

**D1 [JP 10-290699]**

D1 generally relates to manufacturing triglycerides with a high content of γ-linolenic acid (GLA) or dihomo-GLA. (*See* Ex. 1, ¶ 1.) GLA is a polyunsaturated fatty acid containing 18 carbons and three double bonds (i.e., 18:3). D1 discloses triglycerides containing 25 to 36 wt% dihomo-GLA and 28 to 35 wt% GLA. (*See* Ex. 1, ¶19).

The transesterified oil/fat or triglyceride of claim 14 contains:

(1) at least 20% of arachidonic acid,

(2) at least 40% of triglycerides with one residue of arachidonic acid in the molecule, and

(3) no more than 4.0% of AAA.

Likewise, the transesterified oil/fat or triglyceride of claim 11 contains:

(1) at least 20% of PUFAs containing 20 or more carbons and two or more double bonds,

(2) at least 40% of triglycerides with one residue of a PUFA containing 20 or more carbons and two or more double bonds in the molecule, and

(3) no more than 4.0% of triglycerides with 3 residues of the same PUFA containing 20 or more carbons and two or more double bonds.

Arachidonic acid is a 20:4 PUFA, so claim 14 is a species within the genus recited in claim 11.

There is no reason to believe that a process for enriching triglycerides with an 18:3 PUFA would result in 40% of triglycerides having a single 20:2+ PUFA, as recited in claim 11, because the chain length of the PUFA is too low. Instead, the triglycerides would become enriched in triglycerides containing multiple residues of 18:3 PUFAs. Accordingly, the Office has not established even a likelihood of inherency, let alone inherency beyond probabilities or possibilities. *See Oelrich*, 666 F.2d at 581-82. The rejection thus is unsupported and should be withdrawn.

**D2 [Tane et al., 46 J. JPN. OIL CHEM. SOC. 785, (1997)]**

D2 discloses a process of enriching triglycerides in docosahexaenoic acid (DHA, a 22:6 PUFA) and icosapentaenoic acid (EPA, a 20:5 PUFA). D2 obtains a triglyceride with 75% DHA and 15% EPA. There is no reason to believe that the process of D2 would result in 40% of triglycerides having a ***single*** 20:2+ PUFA, as recited in claim 11, because the triglycerides likely contain multiple residues of DHA and EPA.

Further, as the triglycerides in D2 are enriched in DHA and EPA, the proportion of triglycerides having 3 residues of the ***same*** PUFA would be expected to increase, making it less likely that the triglycerides would have less than 4% of triglycerides with 3 residues of the same 20:2+ PUFAs. It is unlikely that the triglycerides disclosed in D2 would meet the recited characteristics of claims 11 (20:2+ PUFAs) or claim 14 (20:4 PUFA). Accordingly, the Office has not established inherency beyond probabilities or possibilities. *See Oelrich*, 666 F.2d at 581-82. The rejection thus is unsupported and should be withdrawn.

**D3 [JP 2000-004894]**

D3 discloses triglycerides containing arachidonic acid and a 20:3 PUFA at positions 1 and 3 of the triglyceride. (*See* Ex. 2, ¶45.) Table 1 shows a gas chromatographic analysis of these triglycerides:

[Table 1]

| 脂肪酸の種類 | 新規構造脂質 | | |
|---|---|---|---|
| | 全体 | 1，3位 | 2位 |
| 8：0 | 9 | 9 | 2 |
| 16：0 | 34 | 6 | 96 |
| 18：1 (n-9) | 11 | 16 | 0 |
| 18：2 (n-6) | 15 | 22 | 1 |
| 18：3 (n-6) | 2 | 3 | 1 |
| 20：3 (n-6) | 1 | 3 | 0 |
| 20：4 (n-6) | 15 | 23 | 0 |

It appears from Table 1 that far less than 40% of the triglycerides have a single 20:2+ PUFA, as opposed to at least 40%, as required by the claims. So D2 does not teach the claim feature that "at least 40% of triglycerides contain one residue of arachidonic acid or a PUFA

containing 20 or more carbons and two or more double bonds in the molecule." D2 does not teach each and every feature of the claimed invention, either explicitly or inherently. *See Verdegaal Bros.*, 814 F.2d at 631. The rejection accordingly should be withdrawn.

## D7 [Liu et al., 75 *JAOCS* 507(1998)]

D7 discloses arachidonic-rich oils from the fungus *Mortierella alpina*. Table 2 of D7 provides a distribution of triglyceride molecular species in the oil:

**TABLE 2**
**Distribution (%, w/w) of Triacylglycerol (TG) Molecular Species in Four Fungal Oils**

| TG species | S1 | S2 | S3 | S4 |
|---|---|---|---|---|
| AAA | 24 | 8 | 5.9 | 10.8 |
| GAA | 1.6 | 0.7 | 0.9 | 0.7 |
| LAA | 7 | 13.3 | 12.7 | 14.2 |
| OAA | 6.2 | 8.5 | 5.9 | 3 |
| PAA | 13.3 | 16.7 | 11.3 | 24.7 |
| SAA | 23 | 12.3 | 9.7 | 15.7 |
| OGA | 0.6 | 0.9 | 0.6 | 0.3 |
| SGA | 0.9 | 0.8 | 0.9 | 0.3 |
| LLA | 0.2 | 0.9 | 1.3 | 0.4 |
| OLA | 0.8 | 3.2 | 3 | 1.1 |
| PLA | 2.4 | 8.4 | 11.5 | 5.1 |
| SLA | 7 | 13.9 | 17.8 | 4.3 |
| OOA | 0.4 | 1.9 | 2.3 | 0.6 |
| PPA | 0.3 | 1.4 | 1.5 | 1 |
| SOA | 2.5 | 4.5 | 5.1 | 0.8 |
| PSA | 0.7 | 1.4 | 1.9 | 0.8 |
| SSA | 0.2 | 0.4 | 0.5 | 0.3 |
| Others[a] | 2.4 | 6.1 | 10.4 | 17.3 |

[a]Nonarachidonic acid-containing TG.

None of the triglycerides disclosed in Table 2 contain no more than 4.0% of triglycerides with 3 residues of the same PUFA containing 20 or more carbons and two or more double bonds. Instead, S1, S2, S3, and S4 contain 24%, 8%, 5.9%, and 10.8% of the species AAA. Accordingly, D7 does not teach each and every element of the claimed invention, either explicitly or inherently. *See Verdegaal Bros.*, 814 F.2d at 631. The rejection accordingly should be withdrawn.

## Akimoto

Akimoto discloses triglycerides containing arachidonic acid and a 20:3 PUFA at positions 1 and 3 of the triglyceride. Table 1 of Akimoto shows a gas chromatographic analysis of these triglycerides:

Table 1

| (units: mol%) | | | |
|---|---|---|---|
| Types of Fatty Acids | Novel Structured Lipids | | |
| | Overall | Positions 1,3 | Position 2 |
| 8:0 | 9 | 9 | 2 |
| 16:0 | 34 | 6 | 96 |
| 18:1 (n-9) | 11 | 16 | 0 |
| 18:2 (n-6) | 15 | 22 | 1 |
| 18:3 (n-6) | 2 | 3 | 1 |
| 20:3 (n-6) | 1 | 3 | 0 |
| 20:4 (n-6) | 15 | 23 | 0 |

It appears that far less than 40% of the triglycerides have a single 20:2+ PUFA, as opposed to at least 40%, as required by the claims. Akimoto thus does not teach each and every element of the claimed invention, either explicitly or inherently. *See Verdegaal Bros.*, 814 F.2d at 631. The rejection accordingly should be withdrawn.

In summary, none of the cited references anticipate the claims under 35 U.S.C. § 102, for the reasons provided above. All the rejections accordingly should be withdrawn and the claims allowed.

## 8. Rejections under 35 U.S.C. § 103(a)

The Office rejects claims 10 and 14 under 35 U.S.C. § 103(a) as allegedly being unpatentable over Akimoto, alone or in view of D1, D2, D3, and D7. The Office alleges that Akimoto teaches the transesterification with 1,3-lipases to produce unsaturated triglycerides which includes arachidonic acid at various positions. Office Action, page 7. The Office further cites *KSR* for the proposition that any difference between the claimed invention and the cited art would have been obvious, absent any explicit analysis of the cited art, simply on the basis that the claimed products are a result of a "combination of familiar elements [made] according to know methods [and yielding] predictable results." Office Action, page 8.

Whether a claim is obvious is based on an objective analysis of the scope and content of the prior art, the differences between the prior art and each element of the claimed invention, and the level of skill in the pertinent art. *See Graham v. John Deere Co.*, 383 U.S. 1, 15-17 (1966). The Board recently addressed an obviousness rejection, where the *KSR* decision was cited. *See*

*Ex parte Whalen*, 89 USPQ2d 1078, 1084 (Bd. Pat. App. & Int. 2008) (precedential). The Board reversed, because the examiner had not provided an apparent reason to combine the cited art in the fashion claimed.

As detailed above, none of the cited references teach or suggest a transesterified oil/fat or triglyceride having the claimed features. Some references may teach one feature, and some references may teach another, but the Office provides no apparent reason why the artisan would have combined the references to achieve the claimed transesterified oil/fat or triglyceride. No *prima facie* case of obviousness can be established without the Office providing an apparent reason to modify the references. *See Whalen*, 89 USPQ2d at 1084. It follows that the Office has not established a *prima facie* case of obviousness.

Only when a *prima facie* case of obviousness has been made does Applicant have a burden of presenting rebuttal evidence. *See In re Piasecki*, 745 F.2d 1468, 223 U.S.P.Q. 785 (Fed. Cir. 1984). The rejection is unsubstantiated and thus must be withdrawn.

**9. Remarks Regarding Shimada *et al., J. Am. Oil Chem. Soc'y* 77: 89-93 (2000)**

To expedite prosecution, Applicants provide the following remarks regarding Shimada *et al., J. Am. Oil Chem. Soc'y* 77: 89-93 (2000) ("Shimada"). Shimada discloses triglycerides in a transesterified oil. Shimada identifies the components, which are depicted in FIG. 3. The text accompanying FIG. 3 states that the amount of AAA (peak 1 of Figure 3) represents 7.3 % wt of the triglycerides. (*See* Shimada, p. 91, right col., first paragraph.) So the triglycerides disclosed in Shimada do not possess the claimed feature of a transesterified oil/fat or triglyceride containing no more than 4.0% of triglycerides with 3 residues of the same PUFA containing 20 or more carbons and two or more double bonds.

## CONCLUSION

The claims are believed in condition for allowance in view of the above arguments, which is respectfully requested. Should the Office have any questions or comments regarding Applicants' amendments or response, Applicants' undersigned representative can be reached at (202) 842-8862. Please direct all correspondence to the below-listed address.

In the event that the Office believes that there are fees outstanding in the above-referenced matter and for purposes of maintaining pendency of the application, the Office is authorized to charge the outstanding fees to Deposit Account No. 50-0573. The Office is likewise authorized to credit any overpayment to the same Deposit Account Number.

Respectfully submitted,

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# EXHIBIT 1

Machine translation of JP H10-290699 (D1):

* NOTICES *



1.This document has been translated by computer. So the translation may not reflect the original precisely.
2.**** shows the word which can not be translated.
3.In the drawings, any words are not translated.

## DETAILED DESCRIPTION

[Detailed Description of the Invention]
[0001]
[Field of the Invention]This invention relates to the manufacturing method of gamma-linolenic acid advanced content triglyceride and/or dihome-gamma-linolenic acid advanced content triglyceride.
[0002]
[Description of the Prior Art]In recent years, the physiology activity which higher unsaturated fatty acid content TORIGU ceride has attracts attention. It is known that especially gamma-linolenic acid content triglyceride and dihome-gamma-linolenic acid content triglyceride have many bioactive operations, such as an improving action, a carcinostatic operation, an immunostimulatory action to adult diseases, such as atopic dermatitis, arthritis-chronica rheumatism, and hypertension. And various examination about the directions to the drugs of gamma-linolenic acid content triglyceride or dihome-gamma-linolenic acid content triglyceride and a food for specified health use is made.
[0003]Conventionally, the loss of a higher unsaturated fatty acid is lessened, and the method of manufacturing the triglyceride which contains a higher unsaturated fatty acid in high concentration is demanded, without carrying out the byproduction of the diglyceride. For example, in JP,63-273485,A. polyunsaturated-fatty-acid content fats and oils and saturated fatty acid -- and -- or saturated fatty acid alcohol ester using specific lipase, Carry out an ester interchange and the triglyceride which has polyunsaturated-fatty-acid acid at least in 2- is contained 40% of the weight or more,And the process of the oil and fat composition in which all the with a carbon numbers of 16 or more saturated fatty acid content is 50 % of the weight or more is indicated, and in JP,6-287594,A. The manufacturing method of the triglyceride which uses fish oil and oleic acid as a raw material, contains oleic acid at least in 1,3- using the ester exchange reaction using specific lipase, and contains docosahexaenoic acid at least in 2- is indicated. In JP,8-214891,A, the manufacturing method of the fats and oils on which the lipase which acts only on 1 of triglyceride and the ester bond like 3-is made to act under existence of fats and oils and medium chain fatty acid is indicated.

[0004]
[Problem(s) to be Solved by the Invention]However, in a method given in JP,63-273485,A. There is a problem that the specific higher unsaturated fatty acid in triglyceride cannot be condensed highly, and in a method given in JP,6-287594,A. Since the oleic acid used for an ester interchange was equivalent to the average molecular weight of the constituent fatty acids of fish oil, the gamma-linolenic acid content in triglyceride could not be raised, and the yield of triglyceride in formed oil fat was not so high as about 90-mol % to raw material triglyceride. In a method given in JP,8-214891,A, Although the moisture content (0 to 1000%) to the amount of enzymes is mentioned, in the example, as much as 2.5g (12,300 ppm) per 202.5g of systems of reaction of moisture is used, as a result of this invention persons' examining a moisture content, even if it applied this moisture content to gamma-linolenic acid content triglyceride, it became clear that the concentration of the higher unsaturated fatty acid in triglyceride was low, and what is still satisfied does not have profit.
[0005]
[Means for Solving the Problem]In then, fats and oils which contain gamma-linolenic acid content triglyceride and/or dihome-gamma-linolenic acid content triglyceride as a result of this invention persons' inquiring wholeheartedly in light of the above-mentioned circumstances. By making lipase which acts only on 1 of triglyceride, and an ester bond like 3-react under medium chain fatty acid and 30-500 ppm existence of water. Medium chain fatty acid whose molecular weight is smaller than higher unsaturated fatty acids, such as gamma-linolenic acid or dihome-gamma-linolenic acid, is introduced at least into 1 and 3-, By being held as it is, gamma-linolenic acid or dihome-gamma-linolenic acid which exists at least in 2-, triglyceride (below gamma-linolenic acid advanced-content triglyceride.) in which a content of gamma-linolenic acid and dihome-gamma-linolenic acid became large as a result dihome-gamma-linolenic acid advanced content triglyceride -- calling -- yield is good, it succeeds in manufacturing continuously for a long period of time, the preservation stability of fats and oils containing triglyceride obtained further finds out a good thing, and it came to complete this invention.
[0006]Hereafter, this invention is explained in detail. Although fats and oils containing gamma-linolenic acid content triglyceride and/or dihome-gamma-linolenic acid content triglyceride (it is written as raw material triglyceride below) are used in this invention, These fats and oils are what contains triglyceride containing gamma-linolenic acid or dihome-gamma-linolenic acid in constituent fatty acids, For example, fats and oils further extracted from algae, such as chlorella and Spirulina, and fungi of Mortierella besides vegetation, such as oleum rapae, Oenotherae Biennis oil, a black gooseberry oil, and a BORAJI oil, can be mentioned.
[0007]As medium chain fatty acid of this invention, it is a carbon number. Although it is chosen out of fatty acid which has 6-12 pieces and caproic acid, caprylic acid, capric acid, lauryl acid, etc. are mentioned, caprylic acid and capric acid are used preferably.
[0008]As lipase used by this invention, what microorganisms, such as the Rhizopus (Rhizopus) group, a RIZOMU call (Rhizomucor) group, and an Aspergillus (Aspergillus) group, produce, a swine pancreatic lipase, etc. are mentioned, for example. A commercial thing can be used about this lipase. for example, lipase (the Tanabe Seiyaku Co., Ltd. make.) of Rhizopus delemar (Rhizopus delemar) lipase (a product made by Novo NORUTISUKU.) of "TARIPAZE" RIZOMU call MIIHEI (Rhizomucor miehei) Lipase (Amano Pharmaceuticals, "lipase A") of "ribozyme IM" and Aspergillus-niger (Aspergillus niger), etc. are mentioned.
[0009]In this invention, since a moisture content can be set to 0 if fixed lipase is used as this

lipase, it is effective in respect of adjustment of a moisture content of the system of reaction mentioned later.
[0010]As a carrier to fix, although cerite, ion-exchange resin, ceramics, etc. are mentioned, ceramics are used preferably, and although ceramic carrier SM-10 (made by NGK Insulators, Ltd.) is preferred as a kind of ceramics, it is not limited to this. When using fixed lipase, the amount of lipase is 1,000 to 300,000 unit preferably 100 to 2,000,000 unit per 1g of carriers.
[0011]0.1 to 30 % of the weight which contains lipase of the above-mentioned number of units especially as a fixing method of lipase, for example although not limited, Making 1-ag [ 10 ] ceramic carrier preferably suspended to 10-30 ml, and 1.0 to 20% of the weight of protein (lipase) solution [ 1-100 ml of ] agitating loosely preferably. -Add gradually 10-300 ml of acetone, ethanol, or isopropanol cooled at 20--80 **, and make lipase stick to immobilization support. Fractions which precipitated are collected and it dries enough under decompression conditions.
[0012]Next, fixed lipase is activated. In the case of a batch method, activation is the fats and oils / medium chain fatty acid / water of ten to 50 time capacity of immobilized enzyme. [30-40:60-70:1-5 (weight ratio)] 30 ** incubates in inside, making it shake for 40 hours. In the case of a column method, activation is the fats and oils / medium chain fatty acid / water 1-10-times the amount of immobilized enzyme. [30-40:60-70:5-10 (weight ratio)] Mixed liquor is dipped and passage liquid is again added in a column. It is the sum total about this operation, and is 1 - 10 cycle ****. The rate of flow in this case is good without limit, if there is no pressure loss.
[0013]Although the ester interchange of the higher unsaturated fatty acid like 1,3-in raw material triglyceride is carried out to medium chain fatty acid with lipase in a manufacturing method of this invention, as a quantity of fats and oils containing raw material triglyceride in the system of reaction, it is 15 to 50 % of the weight preferably ten to 50% of the weight. As a quantity of medium chain fatty acid in the system of reaction, 50 to 90% of the weight, it is 50 to 85 % of the weight preferably, and as for a weight ratio of the raw material triglyceride / medium chain fatty acid in the system of reaction, 1-10 are preferred, and also they are 1-5.
[0014]An addition in the system of reaction of lipase has four to 80,000 preferred unit to 1 g of reaction mixture, and also is 40 to 8,000 unit. One unit here uses olive oil as a substrate, and shows the amount of lipase required to generate fatty acid of a 1micro mol in 1 minute.
[0015]In this invention, it is characterized [ greatest ] by making it react under 30-500 ppm existence of water in the case of this reaction, and is 50-150 ppm preferably. If an ester interchange becomes difficult to advance in less than 30 ppm and water exceeds 500 ppm, since the stability of an enzyme will worsen and hydrolysis of triglyceride will take place, it is not desirable. Although water is contained in fats and oils containing lipase, medium chain fatty acid, and raw material triglyceride, quantity of total water, It is required to control to be set to 30-500 ppm, and as the method of this control, ** Although a moisture content of each ingredient is measured with a Karl Fischer technique and there are a method of controlling a total moisture content, a method of drying ** reaction component thoroughly and adding water of the specified quantity later, etc. beforehand, since handling of some hygroscopic things, such as powdered lipase, is simple, a method of ** is preferred. A moisture content which fixed lipase holds shall not be included in a moisture content of this invention.
[0016]Although both a batch method and a column method are applicable as a reaction method, since the possibility of a reaction and solid liquid separation are continuously easy for a large quantity, a column method is preferred.

[0017]Although a column method which uses fixed lipase below is explained, it is not limited to this. First, a column is filled up with immobilized enzyme and they are the fats and oils / medium chain fatty acid / water of one to 10 time capacity of immobilized enzyme. [30-40:60-70:5-10 (weight ratio)] As a place of activation of the above-mentioned fixed lipase described with mixed liquor, a total of four cycles are dipped and it is activated. Subsequently, if each moisture content of fats and oils and medium chain fatty acid containing raw material triglyceride dried by vacuum distillation, dehydrating treatment, etc. is measured and there is necessity, as the specified quantity addition of the water is carried out and it becomes a moisture content of 30-500 ppm, it will agitate uniformly, and a reaction stock solution will be produced. this reaction stock solution -- linear velocity 0.5 - 1000 ml/hr -- desirable -- 1 - 10 ml/hr, and the space velocity 0.01-10/hr -- a column is preferably passed by 1 [ 0.1-]/hr. Reaction temperature of 10-60 ** is 15-45 ** preferably.
[0018]After neutralizing superfluous medium chain fatty acid which was made to add and carry out the ester interchange of the alkali to obtained passage liquid, and was produced and which was not exchanged for free fatty acid and considering it as fatty acid salt, water is added, this fatty acid salt is extracted to a water layer, an organic solvent is added, and triglyceride (oil reservoir) is collected. A water layer can also be recycled and used for the system of reaction.
[0019]by the above-mentioned ester synthetic reaction, triglyceride receives raw material triglyceride -- 93-97-mol % -- it can collect and dihome-gamma-linolenic acid can be made into 25 to 36 % of the weight for gamma-linolenic acid in triglyceride to 28 to 35% of the weight.
[0020]In this invention, in a reaction of the above-mentioned lipase, it is preferred to also make vitamin E live together, and it contributes to improvement in the preservation stability of fats and oils containing manufactured triglyceride, handling nature, etc. As this vitamin E, either or mixtures, such as alpha-tocopherol, beta-tocopherol, gamma-tocopherol, and delta-tocopherol, are used, and wheat ****** etc. are mentioned preferably.
[0021]In order that triglyceride manufactured by this invention may not separate at all gamma-linolenic acid or dihome-gamma-linolenic acid mostly contained at least in 2-, Contain highly gamma-linolenic acid or dihome-gamma-linolenic acid, and in a manufacturing method of this invention. When an ester exchange reaction by the above-mentioned column method is performed continuously, a reaction which obtains triglyceride with a recovery rate beyond 95 mol % to raw material triglyceride can be run continuously about 30 to 200 days. Even if it allows fats and oils containing obtained triglyceride to stand at a room temperature for a long period of time, there are few rises of acid value, and preservation stability is good.
[0022]
[Example]Hereafter, an example explains this invention still more concretely. However, this invention is not limited to these examples. That it is with "%" shows peak area % which analyzed fatty acid composition by the gas chromatograph.
Example 1 ceramic-carrier SM-10 the [NGK Insulators, Ltd. make] -- Rhizopus Delmer's (Rhizopus delemar) lipase (the Tanabe Seiyaku Co., Ltd. make.) From the upper bed of a column to the BORAJI oil after fixing "TARIPAZE" 5,000 unit / carrier g8g and stuffing the column (1.5-cm [ in diameter ], 6.2-cm [ in length ], and capacity 10.95$cm^3$) of a cylindrical shape It is wheat germ oil (Eisai Co., Ltd. make, "IMIKKUSU") 0.2 % of the weight] as 22.2% of gamma-linolenic acid in triglyceride, [moisture content content of 200 ppm, and vitamin E. 1:2 (weight ratio) mixture of the caprylic acid (moisture content content of 200 ppm) which is medium chain fatty acid of the carbon number 8 [raw-material triglyceride: The ester interchange successive

reaction was performed at 30 **, teaching caprylic acid =1:2(weight ratio)] by linear velocity 4 ml/hr, and space velocity 0.589 / hr. Passage liquid 3g preparative isolation of the obtained reaction mixture was done the one-day back of a reaction start, and 90 days afterward, it added 1N-sodium hydroxide solution, neutralized, removed the water layer (lower layer) after neglect, carried out hexane extraction of the triglyceride layer (upper layer), removed this hexane, and obtained the glyceride fraction. The ODS column (AM120 S-50, product made by YMC) analyzed this glyceride fraction, and the triglyceride fraction was computed with 0.79 g. Methyl esterification of the obtained triglyceride fraction was carried out with the conventional method, capillary chromatography analyzed the fatty acid composition in triglyceride, and gamma-linolenic acid became 29.4%. [Since the triglyceride 0.79g (29.4% of gamma-linolenic acid) was obtained from 1g of raw material triglyceride (22.2% of gamma-linolenic acid), the recovery rate of triglyceride was 96-mol %.] .These fats and oils 1g were put into the seal test tube, the retention test was done for one month at the room temperature, the acid value before preservation and after preservation was measured by the standard fats-and-oils assay method, the rise of acid value was measured, and it evaluated as follows.
O ... [ ... As for more than 10mgKOH / fats and oils g, the result one day after a reaction start is shown in Table 1, and the result of 90 days after is shown in Table 2. ] 1mgKOH / less than fats-and-oils g O ... 1 - 5mgKOH / less than fats-and-oils g ** ... 5 - 10mgKOH / less than fats-and-oils g x
[0023]In example 2 Example 1, except having used the BORAJI oil which does not contain a wheat germ oil (the Eisai Co., Ltd. make, "IMIKKUSU"), it reacted similarly, and analyzed similarly and content triglyceride was obtained for gamma-linolenic acid at recovery rate % of 94 mol to the raw material BORAJI oil 29.0%. The retention test was done similarly. A result is shown in Tables 1 and 2.
[0024]In example 3 Example 1, instead of caprylic acid, capric acid (moisture content of 200 ppm) was carried out to **** for the said weight, and the appearance, and was evaluated similarly. A result is shown in Tables 1 and 2.
[0025]In example 4 Example 1, used Mortierella extracted oil (16% of dihome-gamma-linolenic acid content), and it was made to react like Example 1 instead of a BORAJI oil, and was similarly estimated as Example 1, and the result was shown in Tables 1 and 2.
[0026]In example 5 Example 2, used Mortierella extracted oil (16% of dihome-gamma-linolenic acid content), and it was made to react like Example 1 instead of a BORAJI oil, and was similarly estimated as Example 1, and the result was shown in Tables 1 and 2.
[0027]In example 6 Example 3, used Mortierella extracted oil (16% of dihome-gamma-linolenic acid content), and it was made to react like Example 1 instead of a BORAJI oil, and was similarly estimated as Example 1, and the result was shown in Tables 1 and 2.
[0028]In comparative example 1 Example 1, used a 10 ppm thing for 10 ppm, the moisture content of caprylic acid was made to react to them like Example 1, the moisture content of the BORAJI oil was similarly estimated as Example 1, and the result was shown in Tables 1 and 2.
[0029]In comparative example 2 Example 1, used a 1000 ppm thing for 1000 ppm, the moisture content of caprylic acid was made to react to them like Example 1, the moisture content of the BORAJI oil was similarly estimated as Example 1, and the result was shown in Tables 1 and 2.
[0030]
[Table 1]
Triglyceride Retention test GLA(%) DGLA(%) TORIGURISEDO raw material After a reaction Raw

material After a reaction Recovery rate (mol %) example 1 22.2 29.4 -- --- 96 O example 2 22.2 29.0 -- ---. 94 O example 3 22.2 29.0 --. --- 96 O example 4 -- -- 16.0. 25.3 95 O example 5 -- -- 16.0 25.0 95 O example 6 -- -- 16.025.0 95 O comparative example 1 22.0 28.5 -- --- 98 O comparative example 2 22.0 28.0 -- --- 90 O[0031]

[Table 2]

Triglyceride Retention test GLA(%) DGLA(%) TORIGURISEDO raw material After a reaction Raw material After a reaction Recovery rate (mol %) example 1 22.2 29.2 -- --- 95 O Example 2 22.2 29.0 -- ---. 94 O Example 3 22.2 29.0. -- --- 96 O Example 4 --. -- 16.0 25.3 95 O Example 5 -- -- 16.0 25.0 95 O example 6 -- -- 16.0 25.0 95 O comparative example 1 22.0 22.2 -- --- 100 O comparative example 2 22.0 23.0 -- --- 75 O[0032]

[Effect of the Invention]In this invention, gamma-linolenic acid content triglyceride and/or dihome-gamma-linolenic acid content triglyceride in the included fats and oils under medium chain fatty acid and 30-500 ppm existence of water, Since the lipase which acts only on the ester bond like 1,3-of triglyceride is made to react, yield is good and the preservation stability of the fats and oils containing the triglyceride which it became possible for this triglyceride to be obtained and for it to be made to react continuously in a column for a long period of time using fixed lipase, and was obtained further is good.

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[Translation done.]

# EXHIBIT 2

Machine translation of JP 2000-004894 (D2):

* NOTICES *



1.This document has been translated by computer. So the translation may not reflect the original precisely.
2.**** shows the word which can not be translated.
3.In the drawings, any words are not translated.

## DETAILED DESCRIPTION

[Detailed Description of the Invention]
[0001]
[Field of the Invention]This invention relates to the manufacturing method of new triglyceride, and relates to the manufacturing method of the triglyceride which has the saturated fatty acid of the carbon numbers 16-18 especially in the 2nd place of triglyceride, and has the unsaturated fatty acid of omega3, omega6, and/or omega9 system in either [ at least ] 1 or the 3rd place.

[0002]
[Description of the Prior Art]The great portion of lipid which we are taking in is neutral fat, and it is a mixture of the triglyceride in which 1 of triglyceride, 2, and various fatty acid carried out the ester bond to the 3rd place. And it is pointed out by the difference in the connecting position of fatty acid that the physiology activity differs, and the lipid (structure lipid) which combined specific fatty acid with the position it was decided that triglyceride would be attracts attention especially these days.

[0003]for example, JP,4-12920,B **** -- good triglyceride of the digestion nature which fatty acid of the carbon numbers 8-14 combined with the 2nd place of triglyceride, and fatty acid whose carbon number is 18 or more combined with 1 and the 3rd place is indicated. 2 - JP,5-87497,B since monoglyceride is considered to be a gestalt which tended to be absorbed by people's living body ****. omega3 which has a physiological function in the 2nd place, or omega -- the triglyceride which combined the higher unsaturated fatty acid 6 system, and combined the saturated fatty acid easily hydrolyzed into 1 and the 3rd place with the enzyme of an alimentary canal is indicated.
[0004]On the other hand, if their eyes are turned to the physiological function of fatty acid, arachidonic acid and docosahexaenoic acid attract attention in recent years. These fatty acid is contained in mother's milk.
A report ("Advances in Polyunsaturated Fatty Acid Research", Elsevier Science Publishers, 1993, pp.261-264) that it is useful for a suckling's growth, There is a report (Proc. Natl. Acad. Sci. USA, 90, 1073-1077 (1993), Lancet, 344, and 1319-1322 (1994)) of being important for

embryonic height or cerebral growth.

[0005]And a recommended intake is released from some public institutions (premature baby: docosahexaenoic acid 20 mg/kg weight / [ the arachidonic acid 60, the docosahexaenoic acid 40; normal-child:arachidonic acid 20, and ] day (WHO-FAO (1994)).). In the several countries in Europe, the modified milk for premature babies which blended as triglyceride the arachidonic acid which already combined with docosahexaenoic acid and carried out fermentation production is marketed. However, it is not taken into consideration about the connecting position of the arachidonic acid of triglyceride, and/or docosahexaenoic acid applied to modified milk.
[0006]The triglyceride structure in people's mother's milk has a high rate which pulmitic acid (16:0) combines with the 2nd place of triglyceride, 1. To the 3rd place, and a higher unsaturated fatty acid. . Or it is thought that the rate which medium chain fatty acid combines is high. (Christie, W.W. (1986) The Positional Distribution of Fatty Acids in Triglycerids. Analysis of Oils and Fats in (Hamilton,) R.J., and Russell, J.B., eds.pp. 313-339, Elsevier Applied Science, and London.
[0007]On the other hand, the structure of the arachidonic acid content triglyceride produced by the fermenting method added to modified milk in order to bring the above-mentioned fatty acid composition close to the presentation of mother's milk, Saturated fatty acid including pulmitic acid combines with 1 and the 3rd place, The rate combined with the 2nd place unsaturated fatty acid highly (J. J. Myher, A. Kuksis, K. Geher, P.W. Park, and D.A Diersen-Schade, Lipids 31, and 207-215 (1996)), It differed from what is considered to be people's mother's milk type clearly. Therefore, development of the structure lipid by which structure is checked clearly which the carbon number combined with the 2nd place (the structure lipid considered to be people's mother's milk type triglyceride structure, i.e., triglyceride), and a higher unsaturated fatty acid or medium chain fatty acid combined with the saturated fatty acid of 16-18, 1, and the 3rd place is desired strongly.
[0008]
[Problem(s) to be Solved by the Invention]Therefore, the structure lipid by which this invention is considered to be a Homo sapiens mother's milk type triglyceride structure, That is, the 2nd place of triglyceride is [ a carbon number ] the saturated fatty acid of 16-18, the unsaturated fatty acid combined with 1 and the 3rd place -- at least -- one -- omega3, omega6, or omega -- new triglyceride which is unsaturated fatty acid 9 system. or -- the 2nd place of triglyceride is [ a carbon number ] the saturated fatty acid of 16-18, and either 1 or the 3rd place is [ a carbon number ] the saturated fatty acid of 4-18 -- another side -- omega3, omega6, or omega -- it is going to provide the manufacturing method of new triglyceride which is unsaturated fatty acid 9 system.
[0009]
[Means for Solving the Problem]A method of manufacturing triglyceride which fatty acid of the carbon numbers 8-14 combined with the 2nd place of triglyceride by 1 and an ester exchange reaction using specific lipase the 3rd place, and fatty acid whose carbon number is 18 or more combined with 1 and the 3rd place is above-mentioned JP,4-12920,B. It is indicated. However, in order for fatty acid of the 2nd place to use as a raw material triglyceride in which a carbon number consists of saturated fatty acid of the carbon numbers 16-18 which increased further and at least for 1 and 3 to perform an ester exchange reaction with unsaturated fatty acid of omega3, omega6, or omega9 system using specific lipase, reaction temperature must be not less than 50

**. This reaction is a reaction which used immobilized enzyme, and, in a carbon number, saturated fatty acid of 16-18 combines it with the 2nd place, In order to manufacture 1 and triglyceride which unsaturated fatty acid of omega3, omega6, and/or omega9 system combined with the 3rd place, if reaction temperature becomes high, in addition to a life of an enzyme becoming short, a danger that a higher unsaturated fatty acid will denaturalize is included.
[0010]Then, a result wholeheartedly studied in order that this invention persons might solve the above-mentioned technical problem, To glyceride which saturated fatty acid of 16-18 has combined, a carbon number at the 2nd place. Lipase which acts on 1 and an ester bond of the 3rd place specifically is made to act, At least one fatty acid of 1 and the 3rd place faces manufacturing triglyceride used as unsaturated fatty acid of omega3, omega6, and/or omega9 system by an ester exchange reaction, Fatty acid of the 2nd place of triglyceride is [ a carbon number ] once saturated fatty acid of 16-18, the melting point 1 and whose fatty acid of the 3rd place are medium chain fatty acid makes it go via it as an intermediate, using triglyceride 45 ** or less as a raw material -- it found out that target triglyceride could be manufactured and this invention was completed.
[0011]
[Embodiment of the Invention]According to this invention, in a carbon number, the saturated fatty acid of 16-18 combines with the 2nd place of triglyceride, either [ at least ] 1 or the 3rd place -- omega3, omega6, and/or omega -- the triglyceride which unsaturated fatty acid combined 9 system, a carbon number uses for the 2nd place as a substrate the triglyceride which the saturated fatty acid of 16-18 combined -- omega3, omega6, and/or omega -- it can manufacture under existence of unsaturated fatty acid or its ester 9 system by the ester exchange reaction by 1 and the lipase which acts on the 3rd place specifically.
[0012]Although a carbon number can mention tripalmitin (1, 2, and all of the 3rd place are pulmitic acid (16:0)), and a tristearin (1, 2, and all of the 3rd place are stearic acid (18:0)) to the 2nd place as triglyceride which the saturated fatty acid of 16-18 combined, for example, When the carbon number of the composition saturated fatty acid of triglyceride is 16 or more, this -- 1 and the 3rd place -- specific lipase, omega3, omega6, or omega -- when unsaturated fatty acid is made to react below 50 ** 9 system in the system of reaction which does not contain an organic solvent, the ester exchange reaction in 1 and the 3rd place hardly progresses, and triglyceride with the target structure is not obtained.
[0013]This originates in the character in which it hardly acts on the fats and oils of a solid state, although lipase acts on liquid fats and oils. Therefore, if the carbon number of the composition saturated fatty acid of triglyceride increases, the melting point needs to make reaction temperature high according to the part and this which become high. For example, when using tripalmitin, although it changes with reaction mixture presentations, a reaction must be performed at 50-70 **. For this reason, inactivation of an enzyme and the denaturation of the unsaturated fatty acid added for the ester interchange pose a problem.
[0014]So, when using triglyceride with these high melting points as a substrate raw material. Before exchanging for the unsaturated fatty acid aiming at 1 and fatty acid of the 3rd place by an ester interchange, The fatty acid combined with 1 of raw material triglyceride, and/or the 3rd place For example, with a carbon number of about eight to 12 medium chain fatty acid or oleic acid like caprylic acid, The ester interchange was carried out to fatty acid with the low melting point of linolic acid etc., and it was shown clearly that it is good to use the triglyceride which reduced the melting point at 45 ** or less as a raw material.

[0015]The higher unsaturated fatty acid once combined with the 1st place or the 3rd place in this method, After that Further 1, since it is hard to cause an ester interchange and the ester interchange of the medium chain fatty acid is preferentially carried out, even if it makes specific lipase act the 3rd place, in a carbon number, by repeating a reaction, the saturated fatty acid of 16-18 combines with the 2nd place of the purpose -- 1 and/or the 3rd place -- omega3, omega6, and/or omega -- it clarified that the yield of the triglyceride which unsaturated fatty acid combined 9 system could also be made to increase.
[0016]In order to clarify the feature of this invention, all the fatty acid combined with triglyceride was the same, and explained to the example the case where it was the saturated fatty acid of the carbon numbers 16-18, but. If not all the fatty acid that carries out an ester bond to triglyceride needs to be the same and the saturated fatty acid of the carbon numbers 16-18 has combined with the 2nd place of triglyceride, What kind of fatty acid of the carbon numbers 4-18 may combine with 1 and the 3rd place, or what kind of combination may be sufficient again, and using as a substrate the triglyceride which can react below 45 ** is included in the technical scope of this invention.
[0017]With the triglyceride which saturated fatty acid combined with the 2nd place. If the saturated fatty acid of the carbon numbers 16-18 has combined with the 2nd place, considering the purpose of this invention, either 1 and the 3rd place -- omega3, omega6, or omega -- unsaturated fatty acid having joined together 9 system, and, the position which has not combined unsaturated fatty acid when these substrates are used -- omega3, omega6, or omega -- unsaturated fatty acid can be introduced in an ester interchange 9 system, and the content of the unsaturated fatty acid of omega3 and omega6 which have been combined with 1 and the 3rd place, and/or omega9 system can be raised.
[0018]For example, the 2nd place as triglyceride which unsaturated fatty acid combined with either 1 and the 3rd place with saturated fatty acid, A KURIPUTEKODENIUMU (Crypthecodenium) group, the Thraustochytrium (Thraustochytrium) group, The fats and oils produced by cultivating the microorganism of the Schizochytrium (Schizochytrium) group, a Ur Kenya (Ulkenia) group, a Japonochytrium (Japonochytorium) group, or a HARIFO tris (Haliphthoros) group can be used.
[0019]1 and 2-dipalmitoyl 3-docosahexanolytriglyceride can be isolated from these, for example, this triglyceride -- a substrate -- 1 -- making specific lipase act the 3rd place -- omega3, omega6, or omega, if an ester interchange is carried out to unsaturated fatty acid or its fatty acid ester 9 system, As mentioned above, since the ester interchange of most docosahexaenoic acid is not carried out, the ester interchange only of the pulmitic acid of the 1st place is carried out. When arachidonic acid is used as unsaturated fatty acid, the triglyceride which docosahexaenoic acid combined with either 1 or the 3rd place, arachidonic acid combined with another side, and pulmitic acid combined with the 2nd place can be manufactured.
[0020]At least 1 of triglyceride and 3 can use specific lipase for this invention as a catalyst, Although not limited in particular, for example The Rhizopus (Rhizopus) group, A RIZOMU call (Rhizomucor) group, the Mucor (Mucor) group,Lipase, a swine pancreatic lipase, etc. which microorganisms, such as a penicillium (Penicillium) group, an Aspergillus (Aspergillus) group, the Humicola (Humicola) group, and a fusarium (Fusarium) group, produce are mentioned. A commercial thing can be used about this lipase.
[0021]For example, lipase of Rhizopus delemar (Rhizopusdelemar) (Tanabe Seiyaku Co., Ltd. make; TARIPAZE), Lipase of RIZOMU call MIIHAI (Rhizomucormiehei) (Novo Nordisk make;

ribozyme IM), Lipase of Aspergillus-niger (Aspergillus niger) (the product made from Amano Pharmaceuticals; lipase A), Lipase of the Humicola RANGI norther (Humicolalanuginosa) (Novo Nordisk make; RIPORAZE), Lipase (the product made from Amano Pharmaceuticals; lipase M) of Mucor Java NIKASU (Mucorjavanicus), lipase of fusarium hetero SUPORAMU (Fusariumheterosporum), etc. are mentioned. the using form of these lipase may use the lipase which it could come out of as it was, and could be used, and was fixed in cerite, ion-exchange resin, a ceramic carrier, etc.

[0022]The moisture content applied to this system of reaction is very important, and when water is not included at all, an ester interchange does not advance, When there are many moisture contents, hydrolysis takes place, the recovery rate of triglyceride falls, or spontaneous acyl group transfer happens in the generated partial glycerides, and the saturated fatty acid of the 2nd place transfers to the 1st place or the 3rd place. Therefore, when immobilized enzyme without absorbed water is used, before performing a main reaction, it is effective if the substrate which activates an enzyme first using the substrate which added water, and has not added water in a main reaction is used. In order to pretreat an enzyme using the substrate which contains 0-1 of the applied amount of enzymes, and 000% (% of the weight) of water in order to be activated by a batch reaction and to be activated with a column method, it is good to pour the substrate of water saturation continuously.

[0023]For example, when activated by a batch reaction using lipase (Tanabe Seiyaku Co., Ltd. make; TARIPAZE) of Rhizopus delemar (Rhizopusdelemar) fixed in cerite or a ceramic carrier, a moisture content is 10 to 200% of the applied amount of enzymes (% of the weight). However, a moisture content required for activation of an ester exchange reaction is greatly influenced by the kind of enzyme to be used, For example, if it is lipase (Novo Nordisk make; ribozyme IM) of RIZOMU call MIIHAI (Rhizomucormiehei), moisture is hardly needed but superfluous water must be removed rather. Removal of superfluous water is good to choose as a substrate the triglyceride which does not block a main reaction, and for immobilized enzyme to hydrolyze this.

[0024]What is necessary is for a reaction condition just to determine suitably the amount of the lipase used in a batch reaction, Lipase of Rhizopus delemar (Rhizopusdelemar) fixed, for example in cerite or a ceramic carrier although not restricted in particular, Or when lipase of RIZOMU call MIIHAI (Rhizomucormiehei) is used, 1 to 30% of cocktails (% of the weight) are optimum dose.

[0025]The ester exchange reaction in a batch reaction is performed by the following methods. namely, the triglyceride which the saturated fatty acid of 16-18 combined with the 2nd place in the carbon number -- omega3, omega6, or omega -- unsaturated fatty acid or its fatty acid ester is added 9 system. As fatty acid ester, methyl ester, ethyl ester, propyl ester, butylester, etc. can be used, for example. As for the triglyceride / fatty acid or the triglyceride / fatty-acid-ester ratio used as a raw material, 1:0. 5-20 are optimum dose. Suitable quantity for this substrate (usual [ 5 ], 000-50, and 000 U/g; in the lipase 1U here.) What is necessary is just to perform 45 ** or less of ester exchange reactions near 30 ** preferably for 2 to 100 hours, at least 1 of being the amount of enzymes which separates fatty acid of 1micromol in 1 minute which activated or dried, and 3 adding specific lipase, and stirring or shaking them using olive oil as a substrate.

[0026]Repeated use of the above-mentioned immobilized enzyme can be carried out. That is, a reaction is continuable by leaving only after-reaction immobilized enzyme in a reactor, and exchanging reaction mixture for the newly prepared substrate. The ester exchange reaction by a column method is good to pour a substrate continuously by per [ enzyme 1g ], and 0.05 - 20

ml/hr. The target triglyceride content can be raised by repeating an ester exchange reaction and performing it. namely, omega3, omega6, or omega9 system -- the bottom of existence of unsaturated fatty acid or its fatty acid ester -- 1 of triglyceride -- making specific lipase act the 3rd place -- fatty acid of 1 and the 3rd place -- omega3, omega6, and/or omega -- the reaction mixture by which the ester interchange was carried out to unsaturated fatty acid 9 system is obtained.

[0027]next -- refining triglyceride by the method of mentioning later from this reaction solution, and using this refining triglyceride as a raw material -- again -- omega3, omega6, or omega -- unsaturated fatty acid or its fatty acid ester performs an ester exchange reaction 9 system. This repetition esterification reaction can raise the target triglyceride content by leaps and bounds, and 2 to 5 times of repeat frequency are preferred.

[0028]In the ester exchange reaction using conventional fixed lipase, the acyl group transfer of the fatty acid combined with the 2nd place of the partial glycerides generated by the hydrolysis reaction which occurs as a side reaction was induced. However, in this invention, the hydrolysis reaction could be suppressed nearly thoroughly, and the generated amount of partial glycerides is a grade 1%, and was able to solve the conventional problem. If the moisture content contained in the substrate is thousands of ppm or less, the hydrolysis which takes place as a side reaction can be disregarded, and it has the feature that it is not necessary to carry out close control of the moisture content contained in a substrate.

[0029]It receives that enzyme activity fell by several use at a reaction in the organic solvent using conventional immobilized enzyme, or a not less than 50 ** reaction, It is also possible for inactivation of an enzyme not to take place, in order to react below 45 ** according to the system of reaction which does not use an organic solvent in this invention, but to use an enzyme continuously 100 days or more at a column reaction tens times or more by a batch reaction.

[0030]By this invention, since the substrate is simple, the triglyceride obtained by a reaction comprises several sorts of molecular species. Then, target triglyceride can be easily isolated with conventional methods, such as liquid chromatography, molecular distillation, flowing-down membrane distillation, and superfractionation, or the combination of those. Triglyceride after the reaction manufactured by this invention, It is the triglyceride which unsaturated fatty acid combined with the 1st place and/or the 3rd place, It exists as a mixture with fatty acid or this fatty acid ester combined with 1 of triglyceride of this triglyceride, an unreacted raw material and unreacted unsaturated fatty acid or fatty acid ester, and the raw material that the ester interchange was carried out and was produced, and/or the 3rd place.

[0031]Then, refining of the triglyceride which unsaturated fatty acid combined with the 1st place of the purpose, and/or the 3rd place, and the saturated fatty acid of 16-18 combined with the 2nd place in the carbon number, It can carry out by removing above-mentioned fatty acid and unreacted unsaturated fatty acid by which the ester interchange was carried out by combining alkali deoxidation, steam distillation, molecular distillation, flowing-down membrane distillation, vacuum superfractionation, column chromatography, solvent extraction or membrane separation, or these.

[0032]the fatty acid which constitutes 1 of the triglyceride obtained by this invention, and the 3rd place -- omega3, omega6, and/or omega -- it consists of unsaturated fatty acid 9 system. concrete -- omega3 system -- as unsaturated fatty acid -- 9, 12, and 15-octadecatrienoic acid [ (alpha-linolenic acid) ] -- [18:3, omega3]. 6,9, 12, 15 - Octadeca tetraenoic acid (steer RIDON acid) [18:4, omega3], 11, 14, and 17- eicosatrienoic acid (******- alpha-linoleic acid) -- [20:3,

omega3]. 8, 11, 14, 17-eicosatetraenoic acid [20:4, omega3], 5, 8, 11, 14, 17-eicosapentaenoic acid [20:5, omega3], 7, 10, 13, 16, 19-docosapentaenoic acid [22:5, omega3], 4, 7, 10, 13, 16, and 19- docosahexaenoic acid [22:6, omega3] can be mentioned.
[0033]omega6 system -- as unsaturated fatty acid -- 9 and 12-octadecadienoic acid [ (linolic acid) ] -- [18:2, omega6]. 6, 9, 12-octadecatrienoic acid (gamma- linolenic acid) [18:3, omega6], 8, 11, 14-eicosatrienoic acid (******- gamma-linolenic acid) [20:3, omega6], 5, 8, 11, 14-eicosatetraenoic acid (arachidonic acid) [20:4, omega6], 7, 10, 13, 16-docosatetraenoic acid [22:4, omega6], 4, 7, 10, 13, 16, and - docosapentaenoic acid [22:3, omega6] can be mentioned. [20:3, omega9] 11- eicosatrienoic-acid (mead acid) omega9 system -- as unsaturated fatty acid -- 6, 9- octadecadienoic acid [18:2, omega9], 8, 11-eicosadienoic acid [20:2, omega9], 5, and 8 -- it can mention. An acyl group may be hydroxylation, epoxidation, or an acyl group by which hydroxy epoxidation was carried out. The fatty acid which constitutes the 2nd place of new triglyceride of this invention consists of fatty acid of the carbon numbers 16-18. For example, pulmitic acid (16:0) and stearic acid (18:0) can be mentioned.
[0034]
[Example]Next, an example explains this invention still more concretely. In this example, the following cable addresses show fatty acid and triglyceride for convenience. First, the following are used for the single-character cable address showing fatty acid. 8: Caprylic acid, P:pulmitic acid, A:arachidonic acid, M:mead acid, D : docosahexaenoic acid. Next, it writes by three characters with the single-character cable address showing the fatty acid which has combined triglyceride with the 1st place, the single-character cable address showing the fatty acid combined with the 2nd place, and the single-character cable address showing the fatty acid combined with the 3rd place. Therefore, the structure of triglyceride is written like the following example. Example: 8P8 (triglyceride which caprylic acid combined with caprylic acid at the 1st place, and combined with the 2nd place at pulmitic acid and the 3rd place)
[0035]1:2 (wt/wt) mixture of example 1. tripalmitin (PPP) and caprylic acid is used as a substrate raw material, The reaction mixture which turns into 10.5 g of substrate mixture from 1.2g of fixed RIZOMU call MIIHAI (Rhizomucormiehei) lipase (Novo Nordisk make; ribozyme IM60) is put into a vial bottle with a screw cap, It incubated shaking at 50 ** for 48 hours (a part for 140 times/). After the reaction, it left only immobilized enzyme, reaction mixture was exchanged for new substrate mixture, and it reacted under the same conditions. The reaction was performed 4 times, carrying out repeated use of the immobilized enzyme, and each reaction mixture was collected.
[0036]A 70-ml 0.5N KOH solution (20% ethanol solution) was added to each reaction mixture (10.5g), the evaporator removed the solvent after extracting a glyceride fraction by 100 ml of hexane, and glyceride was collected. As a result of investigating a glyceride presentation by an IYATO loss can (made by YATORON), 8% of diglyceride was contained in the 1st glyceride, but the partial-glycerides content in glyceride of the 2nd henceforth was 1% or less. The fatty acid composition (mol %) of the 2-4th glyceride fractions was 45.1% of caprylic acid, and 54.9% of pulmitic acid.
[0037]In order to raise the replacement factor of caprylic acid, the 2-4th glyceride fractions were used as the raw material, and the ester interchange was carried out again. The glyceride 3.5g and the caprylic acid 7g which were prepared were added to ribozyme IM60 (1.2g) used for the above-mentioned reaction, and it reacted, shaking at 30 ** for 48 hours (the 5th time). After the reaction, reaction mixture was exchanged for a new substrate and it reacted under the same

conditions (the 6th time). Hexane extraction recovered the glyceride fraction from the 5 or 6th reaction mixture (a total of four .8 g). The fatty acid composition (mol %) of the obtained glyceride was 64.2% of caprylic acid, and 35.8% of pulmitic acid. The result which the partial glycerides contained in this glyceride are the following 1%, and was analyzed with the ODS column (Wakosil-II 3C18, 4.6x150 mm, and 2) by using acetone/acetonitrile (1:1, vol/vol) as an elution solvent, The purity of 8P8 was 93%.

[0038]8P8 (3.5g) and 7 g of arachidonic acid (90% of purity) which were obtained were used as the raw material, the ester exchange reaction was performed at 30 ** ribozyme IM60 used for the above-mentioned reaction for 48 hours (the 7th time), hexane extraction of the reaction mixture after a reaction was carried out under alkali conditions, and the glyceride fraction (4.8g) was obtained. When the fatty acid composition of glyceride was analyzed, caprylic acid, pulmitic acid, gamma- linolenic acid, and arachidonic acid were 38.5, 23.1, 2.4, and 34.0-mol %, respectively. The result of having carried out fractionation of this glyceride with the high performance chromatography using an ODS column (SH-345-5, product made from 20 x 500mm YMC) by using acetone/acetonitrile (1:1, vol/vol) as an elution solvent, 8PA and 0.72 or 0.44g of APA were obtained, respectively.

[0039]It reacted on a scale of 100 times of the method indicated in the example 2. example 1, 8P8 was prepared, and it was used as a raw material. Lipase (Tanabe Seiyaku Co., Ltd. make; TARIPAZE) of Rhizopus delemar (Rhizopusdelemar) was fixed in ceramic carrier SM-10 (made by NGK Insulators, Ltd.) according to J. Ferment. Bioeng., 81, and 299-303 (1996). Soybean oil of water saturation after filling up a column with the immobilized enzyme 10g (31, 000 U/g): 100-ml sink immobilized enzyme was activated for caprylic acid 1:2 (wt/wt) mixed liquor by 30 ** and rate-of-flow 3 ml/hr.

[0040]Subsequently, after pouring 50 ml of soybean oil which is not adding water and removing superfluous water, the ester exchange reaction was performed, passing 1:4 (wt/wt) mixture of 8P8 and arachidonic acid ethyl ester (90% of purity) on the same conditions. After distilling 100 g of reaction mixture under the high vacuum and collecting glyceride fractions as residue, according to Example 1, hexane extraction was carried out under alkali conditions. The evaporator removed the solvent and 35.7 g hexane extractable material was obtained. It was 91:9 when the composition ratio of triglyceride and fatty acid ester which are contained in this hexane extractable material was analyzed by the IYATO loss can. As a result of analyzing fatty acid composition, they are caprylic acid, pulmitic acid, gamma-linolenic acid, and ******-. Gamma-linolenic acid and arachidonic acid were 24.4, 34.5, 1.5, 2.6, and 37.0-mol %, respectively.

[0041]In order to remove the superfluous water contained in the fixed RIZOMU call MIIHAI (Rhizomucormiehei) lipase (Novo Nordisk make; ribozyme IM60) used in example 3. example 1, This immobilized enzyme 12g and SUNTGA - The cocktail which consists of 25 (made by Suntory) 60g is put into a 100-ml vial bottle with a screw cap, and it was made to react, shaking at 30 ** for 48 hours (the 1st time). It left only immobilized enzyme to the reactor, and after adding 8P8 (12g) and 48 g of mead acid ethyl ester (90% of purity) which were created in Example 2 and carrying out a nitrogen purge enough, the ester exchange reaction was performed, shaking at 30 ** for 72 hours (the 2 or 3rd time).

[0042]After the reaction, 100 g of them including the 2nd time and the 3rd cocktail was high vacuum distilled like Example 2, and glyceride fractions were collected as residue. Subsequently, after carrying out hexane extraction under alkali conditions according to Example 1, the evaporator removed hexane and a 24.1-g glyceride fraction was obtained. It was 92:8 when the

composition ratio of triglyceride and fatty acid ester which are contained in this was analyzed by the IYATO loss can. High performance chromatography was performed according to Example 1, and MPM was 12.0% about a fixed quantity of fatty acid ester and each triglyceride ingredients from the peak area of a differential refractometer at the bottom and the time. Caprylic acid, pulmitic acid, and the mead acid of the fatty acid composition of this fraction were 31.2, 35.7, and 33.1-mol %, respectively.

[0043]In order to raise a transesterification rate, the ester interchange of the obtained ester interchange triglyceride was again carried out by mead acid ethyl ester. It reacted, having added the ester interchange triglyceride 12g and 48 g of mead acid ethyl ester to the above-mentioned immobilized enzyme, and shaking at 30 ** for 72 hours (the 4th time). It distilled after the reaction by the method which mentioned above 55 g of reaction mixture, and the glyceride fraction of 12.3 g was obtained. Caprylic acid, pulmitic acid, and the mead acid of the fatty acid composition of this fraction were 5.2, 38.6, and 56.1-mol %, respectively.

[0044]In order to remove the superfluous water contained in the fixed RIZOMU call MIIHAI (Rhizomucormiehei) lipase (Novo Nordisk make; ribozyme IM60) used in example 4. example 1, This immobilized enzyme 2g and SUNTGA - The cocktail which consists of 25 (made by Suntory) 10g is put into a 20-ml vial bottle with a screw cap, and it was made to react, shaking at 30 ** for 48 hours (the 1st time). 8P8 (12g) and SUNTGA which left only immobilized enzyme to the reactor and were created in Example 2 - After adding 8 g of fatty acid mixture produced by hydrolyzing 25 and carrying out a nitrogen purge enough, the ester exchange reaction was performed, shaking at 30 ** for 48 hours (the 2-5th time). The glyceride which carried out hexane extraction from the 2-5th cocktails was set after the reaction, and it was considered as the substrate of the ester exchange reaction for the second time.

[0045]They are the ester interchange triglyceride 2g and SUNTGA to the reactor containing the above-mentioned immobilized enzyme. - 10 g of fatty acid mixture of 25 origin is added, and it was made to react, shaking at 30 ** for 48 hours (the 6 or 7th time). The glyceride fraction was extracted from the 6 or 7th cocktail, and it was considered as the substrate of the ester exchange reaction of a third-time degree, and reacted similarly (the 8th time). Gas chromatography analyzed each fatty acid composition, the fatty acid composition which constitutes the triglyceride obtained by repeating an ester exchange reaction 3 times, and triglyceride, of the 1 or 3rd place and the 2nd place. This result is shown in Table 1.

[0046]

[Table 1]

表１（単位：モル%）

| 脂肪酸の種類 | 新規構造脂質 | | |
|---|---|---|---|
| | 全体 | 1，3位 | 2位 |
| 8：0 | 9 | 9 | 2 |
| 16：0 | 34 | 6 | 96 |
| 18：1 (n-9) | 11 | 16 | 0 |
| 18：2 (n-6) | 15 | 22 | 1 |
| 18：3 (n-6) | 2 | 3 | 1 |
| 20：3 (n-6) | 1 | 3 | 0 |
| 20：4 (n-6) | 15 | 23 | 0 |

[0047]8P8 and immobilized enzyme which were created in comparative example 1. example 2 were used as a raw material and a catalyst, respectively. The immobilized enzyme 2g, the soybean oil 4g, the caprylic acid 8g, and the water 0.5g were put into a 20-ml vial bottle, and immobilized enzyme was activated by incubating shaking at 30 ** for 24 hours. It left the activated enzyme in the reactor and the substrate, the arachidonic acid /8P8 which does not contain water in this (4:1, wt/wt), or arachidonic acid/PPP (4:1, wt/wt) was added, and it carried out, having shaken the former reaction at 30 ** and shaking the latter reaction at 50 **. The reaction compared the stability of immobilized enzyme repeatedly, exchanging reaction mixture for the substrate which will seemingly be new every 24 hours.
[0048]When PPP is used for a substrate and a reaction is repeated at 50 **, after using immobilized enzyme 7 times, the uptake quantity of arachidonic acid fell to 10% or less of the first uptake quantity (the uptake quantity of the 1st time and the 7th arachidonic acid is 47% and 3%, respectively). When 8P8 was used for a substrate and a reaction was repeated at 30 ** on the other hand, even if it used immobilized enzyme 50 times, the uptake quantity of arachidonic acid hardly changed (the uptake quantity of the 1st time and the 50th arachidonic acid is 41% and 38%, respectively).

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[Translation done.]